Structurally Novel Bioconversion Products of the Marine Natural Product Sarcophine Effectively Inhibit JB6 Cell Transformation

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Sarcophytol A (**1**) and B (**2**) (see Chart 1) are cembrane-type diterpenes known to inhibit tumor promotion. Indicative of this inhibitory response, we currently report sarcophytol A (**1**) mediates dose-dependent diminution of 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced transformation of JB6 cells. Moreover, a structurally related furanocembrane diterpene, sarcophine (**3**), isolated in good yield from the Red Sea soft coral *Sarcophyton glaucum*, was also found to serve as an effective inhibitor of JB6 cell transformation. This compound was subjected to preparative-scale fermentation with *Absidia glauca* ATCC 22752, *Rhizopus arrhizus* ATCC 11145, and *Rhizopus stolonifer* ATCC 24795, resulting in the production of 10 new metabolites (**5**−**14**) along with the known compound 7β,8α-dihydroxydeepoxysarcophine (**4**). Structures were elucidated primarily on the basis of 2D-NMR spectroscopy, with X-ray crystallography being used to establish the relative stereochemistry of metabolite **5**. When evaluated for potential to inhibit TPA-induced JB6 cell transformation, several of the metabolites mediated inhibitory responses greater than sarcophytol A (**1**) or sarcophine (**3**), most notably 7α-hydroxy-$\Delta^{8(19)}$-deepoxysarcophine (**6**), which was comparable to 13-*cis*-retinoic acid. These studies provide a basis for further development of novel furanocembranoids as anticancer agents.

Sarcophytol A (**1**) and B (**2**) are structurally simple cembranoids isolated from the Okinawan soft coral *Sarcophyton glaucum* (Alcyonaria, Alcyoniidae). These compounds are of interest due to their potential of inhibiting the process of tumorigenesis.[1,2] For example, without toxicity, **1** inhibits spontaneous hepatomas in C3H/HeNCrj mice[3] and *N*-methyl-*N*-nitrosurea-induced large bowel cancer in rats, probably through antipromotional mechanisms.[4] In addition, both **1** and **2** inhibit teleocidin-induced tumor promotion in two-stage carcinogenesis experiments conducted with mice.[5,6] Further, with **1**, pancreatic carcinogenesis induced by *N*-nitrosobis(2-oxypropyl)amine is blocked by antipromotion and antiprogression processes in hamsters,[7,8] and the growth of transplanted human pancreatic cancer cells in nude mice is suppressed.[7]

Although comprehensive SAR studies have yet to be performed, some indications of the mode of inhibitory activity mediated by **1** have been provided. In particular, inhibition of the okadaic acid-induced pathway of tumor promotion by **1** has been associated with reduced expression of protein phosphatases 1 and 2, which in turn leads to an increase in protein phosphorylation and subsequent cell proliferation and induction of TNF-α.[9] Protein isoprenylation was not reduced.[10] A number of 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-mediated responses are also inhibited by **1**, such as lens opacity, oxidative stress, and oxidation of nucleic acids.[11,12] Similarly, in Sencar mouse epidermis exposed to TPA, **1** suppressed oxidant formation, in addition to decreasing infiltration of neutrophils, the level of myeloperoxidase, thymidine glycol, 8-hydroxyl-2′-deoxyguanosine, and 5-hydroxymethyl-2′-deoxyuridine. It also alleviated TPA-induced inflammation and infiltration of phagocytes.[13]

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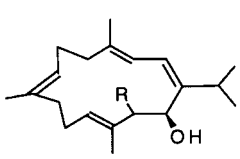
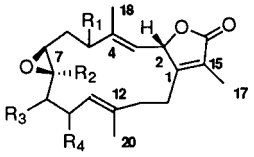
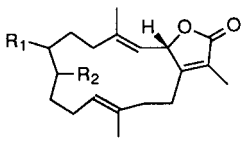
Chart 1



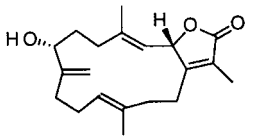
	R
1	H
2	β-OH



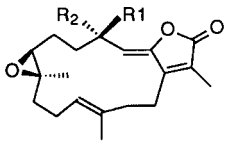
	R_1	R_2	R_3	R_4
3	H	CH_3	H	H
7	H	CH_3	α-OH	H
10	H	CH_2OH	H	H
11	H	CH_3	β-OH	H
12	β-OH	CH_3	H	H
13	H	CH_3	H	β-OH
14	H	CH_3	H	α-OH



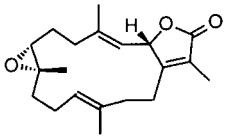
	R_1	R_2
4	β-OH	α-OH, β-CH_3
5	α-OH	β-OH, α-CH_3



6



	R_1	R_2
8	CH_3	OH
9	OH	CH_3



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Thus, a variety of mechanisms appear to contribute to the suppression of tumor promotion by **1**.

In 1974, working with *S. glaucum*, Kashman and coworkers reported the isolation of a related furanocembranoid diterpene, sarcophine (**3**), with remarkable yields of up to 3% dry weight.[14] This substance is a toxin[15] that constitutes the major chemical defense against natural predators.[16] However, unlike **1** and **2**, this readily available cembrane-type diterpene has not been investigated for potential to inhibit tumorigenesis. Thus, we currently report inhibitory potential assessed with cultured JB6 cells, a relevant model system for investigating neoplastic transformation.[17] In addition, to study metabolism that correlates with that of humans,[18] and to produce additional analogues of potential value as anticancer agents, sarcophine was subjected to biotransformation experiments involving various fungal species. This constitutes the first metabolism study reported with a cembranoid diterpene, and some of the novel metabolites were effective inhibitors of JB6 cell transformation.

Results and Discussion

Bioconversion Studies and Structure Elucidation. Sarcophine (**3**) was isolated from the lipophilic extract of the Red Sea soft coral *S. glaucum* in a 1% yield based on dry weight. Twenty-five growing cultures of bacteria and fungi were screened for potential to biconvert **3** to active metabolites. Most cultures were able to convert **3** to more polar metabolites. Of these, *Absidia glauca* ATCC 22752, *Rhizopus arrhizus* ATCC 11145, and *Rhizopus stolonifer* ATCC 24795, were selected for preparative-scale fermentation.

A. glauca ATCC 22752 was able to completely convert **3** to four more polar compounds (**4**−**7**). The NMR spectra of **4** and **5** indicated cleavage of the epoxy function with the addition of one water molecule. The HRFABMS data suggested the molecular formula $C_{20}H_{30}O_4$ and six degrees of unsaturation for **4** and **5**. The IR spectra ($CHCl_3$) of **4** and **5** showed absorption bands at 3592 and 3604 cm^{-1}, respectively, suggesting the presence of a hydroxy functionality. The physical and spectral data of **4** were nearly identical to the previously reported 7*β*,8α-dihydroxydeepoxysarcophine.[19,20] The proton signals resonating at *δ* 3.66 and 3.47 (Table 1) indicate a newly oxygenated methine C-7 in **4** and **5**. The downfield-shifted methyl carbon (C-19) at *δ* 26.4 and 24.2, in **4** and **5**, respectively (Table 1), supported the location of this methyl group on the quaternary carbon C-8, bearing a hydroxy group. Further support is provided by the ^2J and ^3J-HMBC correlations between the C-19 methyl group and C-7, C-8, and C-9 in both compounds.

To determine the relative stereochemistry of **4** and **5**, the latter was subjected to X-ray crystallography. The X-ray crystal structure of **5** (Figure 1) was nearly identical to **3** except in the C-7/C-8 segment. The C(7)−C(8) bond is lengthened by the lack of epoxide functionality in **5**, to 1.55(1) Å as compared with 1.465(4) Å in **3** with a highly strained epoxide ring and bond angles of 58.9(2)−60.7(2)°. The presence of an epoxide group in **3** forces the functional groups on C-7 and C-8 to be eclipsed and apparently forces the methylene groups of C-6 and C-9 to be in an anti-configuration around the C(7)−C(8) bond. Cleavage of the epoxide in **5** causes the molecule to twist, resulting in the more stable staggered conformation in which C-6 and C-9 methylenes are positioned on the same side of the C(7)−C(8) bond (Figure 1). Hence, compound **5** was shown to be 7α,8*β*-dihydroxydeepoxysarcophine.

Bernstein et al. suggested the unique nature of the 14-membered ring macrocycle of **3**.[14] The existence of the segment C7−C11 in a half-chair conformer, based on X-ray crystallography and NOE difference data, restricts the freedom of mobility in the entire macrocycle.[14] This was further supported by the high *J* and dihedral angle values between H-2 and H-3 (10.5 Hz and ∼160°) in **3**, in addition to the paramagnetic deshielding of C-18 methyl group by the C-2 oxygen.[14] This is true whenever the epoxy group is intact; hence, the relative stereochemisty of **7**−**14** could be established on the basis of NOESY spectra. Compound **4** was prepared by an acid-catalyzed transannular reaction of **3**[19] and has been recently reported from *S. trocheliophorum*.[20]

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Table 1. ^{13}C and ^{1}H NMR Spectra of Metabolites 4−7[a]

	4		**5**		**6**		**7**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	163.0, s		163.2, s		162.7, s		161.7, s	
2	79.1, d	5.55, dq (10.1, 1.3)	79.3, d	5.57, dq (13.6, 2.4)	78.7, d	5.61, dq (9.0, 1.9)	78.4, d	5.59, dq (11.6, 1.6)
3	121.2, d	4.93 d (10.2)	120.6, d	4.92, d (13.7)	121.4, d	5.00, d (10.2)	120.7, d	5.02, d (10.1)
4	143.3, s		144.2, s		143.6, s		143.8, s	
5	35.3, t	2.44, ddd (13.8, 13.1, 3.3); 2.20, m	35.3, t	2.43 ddd (17.0, 16.8, 4.2); 2.14, m	36.0, t	2.44, ddd (13.8, 12.8, 3.8); 2.16, m	37.4, t	2.43, m; 2.38, m
6	27.8, t	2.09, m; 1.58, m	26.7, t	1.84, m; 1.55, m	32.1, t	2.06, m; 1.41, m	24.4, t	1.78, m; 1.62, m
7	72.3, d	3.66, dd (9.6, 9.5)	72.5, d	3.47, d (13.5)	70.5, d	4.08, d (10.4)	61.5, d	2.66, dd (5.1, 3.4)
8	78.0, s		75.4, s		154.5, s		60.2, s	
9	38.9, t	2.35, m; 1.95, m	36.8, t	1.81, m; 1.73, m	33.8, t	2.34, m; 2.02, m	78.6, d	2.95, dd (11.8, 4.1)
10	24.8, t	2.28, m; 2.25, m	23.5, t	2.21, m; 2.18, m	29.9, t	2.23, m; 2.18, m	31.6, t	2.49, ddd (14.1, 10.0, 4.1); 2.10, m
11	124.0, d	5.10, dd (6.7, 6.6)	125.2, d	4.98, m	125.8, d	5.11, m	119.1, d	4.93, dd (10.5, 5.1)
12	134.7, s		134.5, s		135.0, s		138.5, s	
13	36.2, t	2.13, m; 2.08, m	36.4, t	2.06, m; 2.00, m	36.8, t	2.19, m; 2.12, m	36.6, t	2.13, m; 1.99, m
14	25.8, t	2.56, m; 2.20, m	26.8, t	2.70, m; 2.11, m	26.3, t	2.71, m; 1.91, m	27.8, t	2.85, ddd (11.3, 8.7, 7.2); 1.98, m
15	122.7, s		122.4, s		122.9, s		123.0, s	
16	174.9, s		175.1, s		174.8, s		177.4, s	
17	8.9, q	1.83, brs	8.8, q	1.80, brs	8.9, q	1.83, s	8.9, q	1.84, t (2.1)
18	15.9, q	1.87, s	16.4, q	1.90, s	16.0, q	1.88, s	16.3, q	1.90, s
19	26.4, q	1.54, s	24.2, q	1.18, s	110.0, t	5.07, brs; 4.93, brs	10.7, q	1.31, s
20	15.7, q	1.66, s	15.2, q	1.68, s	15.3, q	1.62, s	15.3, q	1.68, s

[a] In $CDCl_3$, at 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. Carbon multiplicities were determined by DEPT135 experiments. Abbreviations: s = quaternary, d = methine, t = methylene, q = methyl carbons. Coupling constants (*J*) are in Hz.

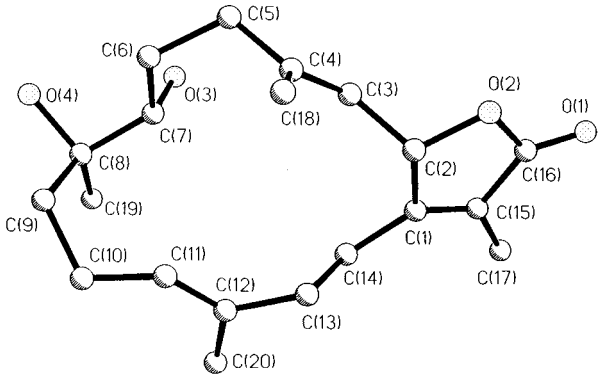

Figure 1. X-ray crystal structure of **5**.

The HRFABMS data for **6** suggested the molecular formula $C_{20}H_{28}O_3$ and seven degrees of unsaturation. The IR spectrum of **6** ($CHCl_3$) showed absorption bands at 3350, 1410, and 905 cm^{-1}, suggesting the presence of hydroxyl and an *exo*-methylene group. This is further supported by the NMR data that also indicated the lack of epoxide functionality and displayed signals for only three methyl groups (Table 1). The olefinic methylene carbon resonating at 110.0 ppm correlated to the two broad singlets at δ 5.07 and 4.93 assigned as the C-19 *exo*-methylene. This is confirmed through the ^{2}J-HMBC coupling of the C-19 methylene protons with the quaternary olefinic carbon at 154.5 ppm. The C19-methylene protons also show ^{3}J-HMBC correlations with the C-9 methylene carbon (33.8 ppm) and the C-7 oxygenated methine carbon at δ 70.5, which correlated to the proton doublet resonating at δ 4.08. The relative stereochemistry of C-7 in **6** was assigned similar to that of **4** and **5** on the basis of a comparison of the splitting and coupling of H-7. It is worth noting that the formation of $\Delta^{8,19}$ in **6** was directed according to Hofmann's rule, toward the least highly substituted carbon C-19 and not toward the more substituted C-9 to form the less stable Hofmann product.[21] Compound **6** proved to be 7α-hydroxy-$\Delta^{8(19)}$-deepoxysarcophine. The 7*β*-epimer of **6** was previously obtained as an oil by treating **3** with 2% H_2SO_4 in acetone.[19] This latter compound showed different physical properties from **6**; however, the spectral data are very similar.

The HRFABMS data for **7** and **11** suggested the molecular formula $C_{20}H_{28}O_4$ and seven degrees of unsaturation. IR spectra of **7** and **11** display absorption bands at 3420, 1230, 880 cm^{-1} (**7**) and 3480, 1236, 880 cm^{-1} (**11**) suggesting the presence of hydroxyl and epoxy functionalities. The NMR data of both compounds indicated they are 9-hydroxy derivatives of sarcophine (Tables 1 and 2). The proton doublet of doublets resonating at δ 2.95 and 3.92 correlate to the oxygenated methine carbons (C-9) at δ 78.6 and 70.7 of **7** and **11**, respectively. This is further supported by the ^{3}J-HMBC couplings of **7** and **11** C-9 protons with the C-19 methyl singlet and the oxirane methine (C-7). The relative stereochemistry of both compounds was assigned on the basis of NOESY correlations. A strong NOESY correlation between H-9 of **11** with the 19α-methyl group and H-2α proton suggested that it is in α orientation. This is also supported by the paramagnetic shielding of the H-9 in **7** by the epoxy and $\Delta^{11,12}$ as compared to the same proton of **11**. Hence, compounds **7** and **11** proved to be 9α- and 9*β*-hydroxysarcophine, respectively.

The HRFABMS spectra of **8** and **9** suggested the molecular formula $C_{20}H_{28}O_4$ and seven degrees of unsaturation. The IR spectra of **8** and **9** suggested the presence of hydroxy (3434 and 3409 cm^{-1} in **8** and **9**, respectively) and epoxy (1230, 875 cm^{-1} in **8** and 1238, 880 cm^{-1} in **9**) groups. The NMR spectra of **8** and **9** suggested that both are stereoisomers in which $\Delta^{3,4}$ migrated to $\Delta^{2,3}$ and hydroxylation occurred at C-4 (Table 2). The olefinic proton singlet resonating at δ 5.40 and

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Table 2. ^{13}C and ^{1}H NMR Spectra of Metabolites 8−11[a]

	8		9		10		11	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	151.9, s		151.9, s		162.1, s		162.0, s	
2	148.2, s		147.8, s		78.8, d	5.55, dq (10.9, 1.0)	78.3, d	5.57, dq (10.0, 1.5)
3	116.1, d	5.40, s	116.7, d	5.44, s	120.8, d	5.04, dq (10.8, 1.0)	120.7, d	5.03, dq (10.1, 1.0)
4	73.5, s		72.5, s		143.7, s		143.4, s	
5	39.5, t	1.96, m; 1.51, m	39.8, t	2.06, m; 2.00, m	37.4, t	2.37, m; 2.33, m	37.2, t	2.40, m; 2.38, m
6	22.7, t	2.09, m; 1.64, m	22.8, t	1.98, m; 1.61, m	24.6, t	2.08, m; 1.98, m	24.5, t	1.97, m; 1.72, m
7	60.8, d	2.60, m	61.6, d	2.59, m	61.5, d	2.85, dd (4.6, 4.5)	57.1, d	2.96, dd (4.2, 4.1)
8	61.4, s	-	60.5, s		62.4, s		61.2, s	
9	35.7, t	2.58, m; 1.82, m	35.0, t	2.68, dd (10.5, 5.1); 1.79, m	33.6, t	2.38, m; 1.24, m	70.7, d	3.92, dd (4.8, 2.1)
10	23.4, t	2.07, m; 2.00, m	24.0, t	1.69, m; 1.63, m	22.7, t	1.94, m; 1.78, m	29.0, t	2.59, m; 2.17, m
11	128.4, d	5.01, dd (7.5, 7.0)	128.1, d	4.99, dd (7.3, 6.8)	125.1, d	5.15, dd (9.1, 5.6)	118.1, d	4.96, dd (10.6, 4.7)
12	131.3, s		131.2, s		135.7, s		138.7, s	
13	37.8, t	2.37, m; 2.25, m	37.2, t	2.35, m; 2.24, m	36.3, t	2.07, m; 1.94, m	36.5, t	2.11, m; 2.03, m
14	22.9, t	2.55, m; 1.46, m	22.9, t	2.56, m; 1.74, m	27.5, t	2.70, m; 2.12, m	27.6, t	2.84, m; 2.00, m
15	124.0, s		123.9, s		123.0, s		122.5, s	
16	169.8, s		169.9, s		174.7, s		174.4, s	
17	9.0, q	1.95, s	9.0, q	1.95, brs	9.0, q	1.86, brs	8.6, q	1.82, t (1.4)
18	30.5, q	1.50, s	29.7, q	1.52, s	16.1, q	1.88, brs	15.8, q	1.89, d (1.2)
19	18.3, q	1.21, s	18.7, q	1.24, s	62.5, t	3.85, d (11.9); 3.63, d (11.9)	15.7, q	1.29, s
20	15.8, q	1.57, s	16.4, q	1.56, s	15.5, q	1.62, s	15.4, q	1.67, s

[a] In $CDCl_3$, 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. Carbon multiplicities were determined by DEPT135 experiments. Abbreviations: s = quaternary, d = methine, t = methylene, q = methyl carbons. Coupling constants (*J*) are in Hz.

Table 3. ^{13}C and ^{1}H NMR Spectra of Metabolites 12−14[a]

	12		13		14	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	161.6, s		162.3, s		159.0, s	
2	78.1, d	5.60, d (9.7)	79.1, d	5.50, dq (9.9, 1.4)	79.0, d	5.55, dq (9.3, 1.2)
3	123.4, d	5.22 dq (9.9, 1.0)	120.7, d	5.04, dq (9.7, 0.9)	117.5, d	5.04, d (9.6)
4	145.3, s		143.6, s		143.9, s	
5	76.1, d	4.38, dd (11.6, 3.6)	37.0, t	2.36, m; 2.28, m	36.4, t	2.26, m; 2.17, m
6	33.1, t	2.15, m; 1.76, ddd (15.1, 4.5, 4.0)	25.5, t	2.32, m; 1.76, m	25.4, t	1.97, m; 1.55, m
7	58.7, d	2.51, dd (4.4, 3.9)	61.6, d	2.80, dd (5.7, 3.8)	58.6, d	2.79, dd (5.6, 3.3)
8	59.7, s		59.2, s		59.8, s	
9	38.8, t	2.12, m; 1.09, ddd (13.3, 12.7, 2.0)	45.5, t	2.29, dd (13.7, 3.0) 1.64, dd (13.8, 9.0)	44.9, t	2.19, m; 1.53, dd (14.5, 2.4)
10	27.6, t	1.26, m; 1.25, m	66.0, d	4.46, ddd (8.8, 8.6, 3.0)	65.5, d	4.55, ddd (9.3, 6.7, 2.5)
11	125.0, d	5.15, dd (9.8, 5.2)	127.9, d	5.18, dq (8.3, 1.2)	129.2, d	5.24, d (9.2)
12	135.5, s		136.9, s		138.0, s	
13	36.5, t	2.18, m; 2.01, m	35.1, t	2.19, m; 2.17, m	37.1, t	2.37, m; 2.28, m
14	23.2, t	2.75, ddd (11.5, 10.1, 7.6); 2.04, m	24.6, t	2.55, m; 1.60, m	27.6, t	2.62, m; 1.62, m
15	123.3, s		123.3, s		120.5, s	
16	177.0, s		174.6, s		178.2, s	
17	9.0, q	1.86, brs	9.1, q	1.85, t (1.5)	9.3, q	1.89, t (1.6)
18	10.8, q	1.94, brs	16.1, q	1.80, d (1.3)	16.4, q	1.91, brs
19	17.3, q	1.25, s	18.8, q	1.28, s	20.2, q	1.37, s
20	15.4, q	1.61, s	17.1, q	1.71, s	16.1, q	1.66, s

[a] In $CDCl_3$, 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. Carbon multiplicities were determined by DEPT135 experiments. Abbreviations: s = quaternary, d = methine, t = methylene, q = methyl carbons. Coupling constants (*J*) are in Hz.

5.44 which correlate to methine carbons 116.1 and 116.7 ppm in **8** and **9**, respectively, are assigned as H-3. This proton displayed ^{2}J-HMBC couplings to the oxygenated quaternary olefinic carbons (C-2) at 148.2 ppm in **8**. The proton H-3 in **8** also displayed ^{3}J-HMBC correlation with the quaternary olefinic β-unsaturated carbons resonated at 151.9 ppm (C-1). The methyl carbons (C-18) resonating at 30.5 and 29.7 ppm in **8** and **9** suggested its location on a quaternary hydroxylated carbon. This is further supported by the HMBC correlation of the C-18 proton signal with the quaternary oxygenated carbon C-4 (δ 73.5) in **8**. The relative stereochemistry of C-4 was again determined with the aid of NOESY spectra. The exchangeable proton singlet absorbed at δ 2.85 which was assigned as the C-4 hydroxy group showing a strong NOESY correlation with the 19α-methyl group in **8**, which suggest the same relative stereochemistry for both groups. The strong NOESY correlation between the 18- and 19-methyl groups suggested similar relative stereochemistry for both groups in **9**.

The HRFABMS and IR data of **10** indicated similar molecular formula and functionality as **8** and **9**. The NMR spectra of **10** indicated the hydroxylation of the C-19 methyl group (Table 2). The two proton doublets resonating at δ 3.85 and 3.63 correlated to the oxygenated methylene carbon at 62.5 ppm and was assigned as the new hydroxymethyl group at C-19. This is further supported by HMBC correlations between quaternary carbon C-8 at 62.4 ppm and with the C-9 methylene group at 33.6 ppm. Compound **10** was shown to be 19-hydroxysarcophine.

The HRFABMS, IR, and NMR data of **12** indicated it is a 5-hydroxysarcophine derivative (Table 3). The doublet of doublet resonating at δ 4.38 which correlated to the oxygenated methine carbon at 76.1 ppm is assigned as C-5. The H-5 proton shows COSY coupling with H-6 proton which in turn is coupled to the oxirane methine proton H-7. The position of H-5 is further confirmed through its ^{3}J-HMBC correlation with the C-18 methyl signal. Once again, the relative stereochemistry of H-5

was assigned α, on the basis of its NOESY correlations with the (C-19)-methyl and H-7 α-protons. Compound **12** was found to be 5β-hydroxysarcophine.

The HRFABMS, IR, and NMR data of **13** and **14** indicated that they are epimeric 10-hydroxysarcophine derivatives (Table 3). The proton signals resonating at δ 4.46 and 4.55 correlated to the oxygenated methine carbons at 66.0 and 65.5 ppm are assigned as H-10 in **13** and **14**, respectively. The proton H-10 in both compounds shows distinct COSY coupling with olefinic H-11, now split as a doublet of quartet (J = 8.3, 1.2) in **13** and as a sharp doublet (J = 9.2) in **14**. Finally, a NOESY spectrum indicates the relative stereochemistry of C-10 in **13** and **14**. The oxygenated proton H-10 of **13** shows NOESY correlations with the C-19 α-methyl group and the H-9 signal resonating at 2.29 ppm indicating its α-orientation. Compounds **13** and **14** proved to be 10β- and 10α-hydroxysarcophine, respectively.

Inhibition of JB6 Cell Transformation. In preliminary studies, sarcyphytol A (**1**) and sarcophine (**3**) were investigated for potential to inhibit phorbol 12,13-dibutyrate binding with partially purified protein kinase C,[22] antioxidant activity based on stabilization of 1,1-diphenyl-2-picrylhydrazyl free radicals,[23] and inhibition of TPA-induced ornithine decarboxylase activity with cultured mouse 308 cells.[24] In each case, no significant activity was observed (IC_{50} values >250, >500 and >50 μM, respectively) (data not shown). Thus, on the basis of the known potential of **1** to inhibit tumor promotion, cultured JB6 cells[25] were used as a model system. JB6 cell lines were originally derived from untreated primary BALB/c mouse epidermal cell cultures that gave rise to a low frequency of immortalized cell lines.[17,25] However, treatment of select JB6 cells with TPA results in the irreversible acquisition of tumorigenecity in nude mice and anchorage-independent growth in soft agar.[25,26] The latter characteristic represents an experimentally assessable end point that is suitable for studying a series of analogues.

Accordingly, sarcophytol A (**1**) (supplied by Dr. H. Fujiki), sarcophine (**3**), its bioconversion products **4**−**14**, and sarcophine 7,8-diastereoisomer (**15**),[27] which was isolated from the same *Sarcophyton* sp., were subjected to anchorage-independent JB6 cell transformation assays. As summarized in Table 5, compounds **1**, **3**, **6**, **9**, **10**, and **15** were effective inhibitors of TPA-induced anchorage-independent growth (ED_{50} values <50 μM). At the highest concentration tested, greatest inhibition was observed with sarcophytol A (**1**), but sarcophine (**3**) and its metabolites were of equal to or greater potency at lower concentrations. Of particular note, at the lowest test concentration (0.4 μM), 7α-hydroxy-$\Delta^{8(19)}$-deepoxysarcophine (**6**) inhibited TPA-induced JB6 colony formation by approximately 46%, while the known cancer chemopreventive agent sarcophytol A (**1**) was only marginally active. Importantly, these test compounds did not mediate significant cytotoxic/cytostatic effects with JB6 cells (IC_{50} values >50 μM). Thus, inhibition of TPA-induced anchorage-independent growth is a specific event, and the potency of some sarcophine metabolites (notably **6**) approaches that of 13-*cis*-retinoic acid.

Since inhibition of TPA-induced JB6 cell transformation shows a good correlation with inhibition of tumorigenesis,[26,27] the test compounds currently described are promising anticancer agents. As noted above, sarcophytol A (**1**) has been reported to demonstrate potent antitumor activity, predominantly at the level of promotion.[5,8] The ready availability of sarcophine (**3**) and the potential of generating derivatives of greater potency through microbial transformation bode well for development.

Table 4. Atomic Coordinates (× 10^4) and Equivalent Isotropic Displacement Paramters (Å^2 × 10^3) of 5[a]

atom	*x*	*y*	*z*	*U*(eq)[a]
C(1)	−1694(7)	1295(7)	6824(6)	57(2)
C(2)	−1714(7)	1856(7)	7874(5)	61(2)
C(3)	−853(7)	1434(7)	8571(6)	67(2)
C(4)	−139(7)	2081(7)	9091(6)	62(3)
C(5)	720(6)	1493(8)	9709(5)	71(3)
C(6)	1832(6)	1645(7)	9234(5)	64(2)
C(7)	1988(7)	917(6)	8257(5)	55(2)
C(8)	3092(6)	1051(7)	7729(6)	58(2)
C(9)	3385(6)	2282(6)	7443(6)	63(2)
C(10)	2855(7)	2786(7)	6470(6)	72(3)
C(11)	1668(7)	2876(6)	6509(6)	59(2)
C(12)	957(8)	2498(6)	5839(6)	58(2)
C(13)	−201(6)	2569(6)	6081(6)	61(2)
C(14)	−684(6)	1354(7)	6211(5)	62(2)
C(15)	−2584(7)	745(7)	6650(7)	64(3)
C(16)	−3272(8)	889(9)	7538(10)	84(3)
C(17)	−2930(6)	65(7)	5745(6)	94(3)
C(18)	−137(6)	3353(6)	9109(6)	81(3)
C(19)	3193(7)	251(7)	6803(5)	83(3)
C(20)	1244(7)	1955(6)	4817(5)	79(3)
O(1)	−4152(5)	514(6)	7681(6)	113(3)
O(2)	−2760(5)	1503(6)	8273(5)	85(2)
O(3)	1833(4)	−265(4)	8505(4)	82(2)
O(4)	3834(5)	680(4)	8536(4)	80(2)

[a] *U*(eq) is defined as one-third of the trace of the orthogonalized U_{ij} tensor.

Experimental Section

General Experimental Procedure. Melting points are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The HRFABMS spectra were conducted at the University of Kansas. TLC analyses were carried out on precoated silica gel G_{254}, using developing system $CHCl_3$−MeOH (19:1), or aluminum oxide ALOX-100 UV_{254}, using developing system cyclohexane−ethyl acetate (2:1). For column chromatography, Si gel 60 of 40 μm was used.

Materials. The Red Sea soft coral *Sarcophyton glaucum* was collected in December 1994 from Hurghada, Egypt, by snorkeling and scuba at 1−3 m. The frozen coral (1.1 kg) was lyophilized and extracted with 95% EtOH (3 × 2 L).

Organisms. Microbial metabolism studies were conducted as previously reported.[28] Twenty-five microbial cultures, obtained from the University of Mississippi, Department of Pharmacognosy culture collection, were used for screening. These microbes were the following: *Absidia glauca* ATCC 22752, *Aspergillus flavipes* ATCC 1030, *Aspergillus ochraceus*

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Table 5. Effects of Sarcophytol A (1), Sarcophine (3), and Derivatives 4−15 on TPA-Induced JB6 Cell Transformation[a]

test compd	concentration tested (μM)			
	0.4	2.0	10.0	50.0
sarcophytol A (**1**)	5.0 ± 11.6	32.2 ± 5.9	32.2 ± 7.3	86.4 ± 7.1
sarcophine (**3**)	13.6 ± 8.1	21.5 ± 11.0	39.7 ± 14.0	58.0 ± 8.5[a]
7α-hydroxy-$\Delta^{8(19)}$-deepoxysarcophine (**6**)	46.1 ± 12.9	42.4 ± 5.5	41.0 ± 5.5	57.1 ± 5.1
metabolite (**9**)	20.6 ± 8.1	34.0 ± 3.6	37.4 ± 2.3	54.9 ± 7.6
19-hydroxysarcophine (**10**)	26.3 ± 6.5	72.8 ± 3.7	51.8 ± 8.3	56.3 ± 3.2
7,8-sarcophine diastereoisomer (**15**)	12.4 ± 18.0	41.9 ± 10.8	65.9 ± 10.4	63.1 ± 3.4

[a] Data are expressed as mean % inhibition SD (triplicate determinations). At a test concentration of 50 μM, 7β,8α-dihydroxydeepoxysarcophine (**4**), 7α,8β-dihydroxydeepoxysarcophine (**5**), 9α-hydroxysarcophine (**7**), metabolite (**8**), 9β-hydroxysarcophine (**11**), 5β-hydroxysarcophine (**12**), 10β-hydroxysarcophine (**13**), and 10α-hydroxysarcophine (**14**) inhibited transformation by 0.5, 11.2, 3.3, 35.9, 37.8, 36.0, 12.6, and 9.9%, respectively. Lower concentrations were not tested. 13-*cis*-Retinoic acid, a positive control, inhibited transformation by 95.9% at 1 μM.

ATCC 18500, *Aspergillus ochraceus* ATCC 22947, *Aureobasidium pullulans* ATCC 9348, *Beauvaria bassiana* UM-ATCC 7159, *Caldariomyces fumago* ATCC 11925, *Calonectria decora* ATCC 14767, *Chaetomium cochliodes* NRRL 2320, *Cladosporium*, 9.5:0.5 *resinae* ATCC 22712, *Coriolus antarcticus* ATCC 34581, *Cryptococcus neoformans* ATCC 32264, *Cunninghamella blakesleeana* ATCC 8688a, *Cunninghamella elegans* ATCC 9245, *Fusarium oxysporium* ATCC 7601, *Fusarium solani* ATCC 12823, *Nocardia species* ATCC 21145, *Penicillium chrysogenum* ATCC 9480, *Rhizopus arrhizus* ATCC 11145, *Rhizopus stolonifer* ATCC 24795, *Saccharomyces cerevisiae* ATCC 2366, *Saccharomyces lipolytica* ATCC 8661, *Streptomyces flocculus* ATCC 25453, *Streptomyces griseus* ATCC 13968, *Streptomyces lavendulae* L-105.

Microbial Metabolism of Sarcophine (3). *A. glauca* ATCC 22752 and *R. stolonifer* ATCC 24795 were grown in five 1 L culture flasks, each containing 300 mL of compound medium α, while *R. arrhizus* ATCC 11145 was grown in six 0.5 L culture flasks, each containing 150 mL. A total of 780.0 mg of **3** was dissolved in 2.5 mL of EtOH and evenly distributed among the stage II (24 h) cultures in a concentration of 60 mg/1 L flask or 30 mg/0.5 L flasks. After 14 days, the incubation mixtures for each organism were pooled and filtered. The filtrates (1.25, 1.3, and 0.6 L, respectively) were exhaustively extracted with EtOAc (3 × 500 mL), which were then dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Each residue (920 mg in *A. glauca*, 860 mg in *R. stolonifer*, and 420 mg in *R. arrhizus*) was flash chromatographed over 50 g of Si gel 60 using hexane, gradient elution with increasing proportions of acetone, and finally MeOH. In the case of *A. glauca*, repeated prep TLC of the less polar fractions on Si gel G (9.5:0.5 $CHCl_3$−MeOH) afforded **4** (15 mg, R_f 0.45) and **6** (11 mg, R_f 0.33). The polar fractions were subjected to prep TLC over C18-RP (5:2 CH_3CN-H_2O) to afford **5** (12 mg, R_f 0.55) and **7** (4 mg, R_f 0.39). Fractions from *R. stolonifer* metabolism were subjected to prep TLC over Si gel G_{254} (19:1 $CHCl_3$−MeOH) to afford **10** (3 mg, R_f 0.41), **11** (12 mg, R_f 0.33), and the epimeric mixture **8** and **9**. This was further chromatographed on Al_2O_3 preparative TLC using cyclohexane−EtOAc (2:1) to afford **8** (6 mg, R_f 0.51) and **9** (5 mg, R_f 0.46). Fractions of *R. arrhizus* metabolism residue were similarly chromatographed over Al_2O_3 to afford **12** (4 mg, R_f 0.66), **13** (2 mg, R_f 0.42), and **14** (1 mg, R_f 0.48).

7β,8α-Dihydroxydeepoxysarcophine (**4**): colorless needles from EtOH, mp 142 °C, $[\alpha]_D^{25}$ +104.6 (*c* 0.26, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 246 (2.87), 268 (2.67) nm; IR ν_{max} ($CHCl_3$) 3592, 3423 (OH), 3027−2856, 1745 (C=O) 1680, 1230 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* calcd for $C_{20}H_{31}O_4$ (M + H)$^+$ 335.2222, found 335.1767.

7α,8β-Dihydroxydeepoxysarcophine (**5**): colorless prisms from EtOH, mp 99 °C, $[\alpha]_D^{25}$ + 2.2 (*c* 0.22, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 247 (2.88), 275 (2.68) nm; IR ν_{max} ($CHCl_3$) 3604, (OH), 3117−2827, 1745 (C=O) 1680, 1230 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* calcd for $C_{20}H_{31}O_4$ (M + H)$^+$ 335.2222, found 335.2215. X-ray crystallography: The colorless crystal 0.5 × 0.5 × 0.5 mm was mounted on a glass fiber, and the unit cell was determined to be orthorhombic using 25 reflections with 2θ from 15 to 30°. Data were collected using Mo Kα radiation by both ω and the $\theta/2\theta$ scan methods from 2θ of 4.5 to 40°. Three check reflections were monitored every 97 reflections, showing no decrease in intensity over the duration of the data collection. The crystal and structure refinement data were the following: temperature 293(2) K, λ 0.710 73 Å, crystal system orthorhombic, space group $P2_12_12_1$; unit cell dimensions a = 12.56(2) Å, α = 90°; b = 11.66(2) Å, β = 90°; c = 12.92(2) Å, γ = 90°, V 1892 (5) Å^3, Z = 4; calculated density 1.178 mg/m^3; absorption coefficient 0.080 mm^{-1}, F(000) 732, θ range for collection 2.26−20.03°, index ranges $0 \le h \le 12$, $0 \le k \le 11$, $0 \le l \le 12$, reflections collected 1039, independent reflections 1037 (R_{int} = 0.0714), refinement method full-matrix least squares on F^2, data/restraints/parameters 991/0/227, goodness-of-fit on F^2 0.915, final R indices [$I > 2\sigma < (I)$] $R1$ = 0.0474, w$R2$ = 0.1067, R indices (all data) $R1$ = 0.0745, w$R2$ = 0.1144, absolute structure parameter 8(4), extinction coefficient 0.017(3), largest difference peak and hole 0.172 and −0.155 eÅ^{-3}. The structure was solved using the direct methods package of SHELX-86.[29] All atoms appeared in the solution following the initial generation of a Fourier difference map. These atoms were included in the atom list and refined by least-squares using SHELX-93.[30] Hydrogen atoms were included at calculated positions and were refined using group thermal parameters. Anisotropic refinement of all non-hydrogen atoms lead to a final R value of 7.45% on all data. Atomic coordinates for all non-hydrogen atoms are listed in Table 4. Figure 1 shows a drawing of crystal structure of **5** with 25% probability thermal parameters.

7α-Hydroxy-$\Delta^{8(19)}$-deepoxysarcophine (**6**): colorless needles from EtOH, mp 94 °C, $[\alpha]_D^{25}$ +4.9 (*c* 0.13, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 246 (2.81), 275 (2.64) nm; IR ν_{max} ($CHCl_3$) 3350, (OH), 3170−2811, 1797 (C=O) 1691, 1682, 1410, 1383, 905 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* calcd for $C_{20}H_{29}O_3$ (M + H)$^+$ 317.2117, found 317.2115.

9α-Hydroxysarcophine (**7**): colorless needles from EtOH, mp 86 °C, $[\alpha]_D^{25}$ −3.2 (*c* 0.10, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 247 (2.52), 278sh (2.03); IR ν_{max} ($CHCl_3$) 3420, (OH), 3022−2854, 1747 (C=O) 1598, 1230, 880 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* calcd for $C_{20}H_{29}O_4$ (M + H)$^+$ 333.2066, found 333.2068.

Metabolite **8**: colorless needles from EtOH, mp 108 °C, $[\alpha]_D^{25}$ −5.0 (*c* 0.10, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 247 (2.65), 289 (2.51), 298 (2.45) nm; IR ν_{max} ($CHCl_3$) 3434, (OH), 3018−2852, 1758 (C=O) 1680, 1230, 875 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRFABMS *m/z* calcd for $C_{20}H_{29}O_4$ (M + H)$^+$ 333.2066, found 333.2045.

Metabolite **9**: colorless needles from EtOH, mp 102 °C, $[\alpha]_D^{25}$ −1.4 (*c* 0.10, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 247 (2.71), 287 (2.49), 295 (2.39) nm; IR ν_{max} ($CHCl_3$) 3409, (OH), 3018−2820, 1760 (C=O) 1658, 1238, 880 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRFABMS *m/z* calcd for $C_{20}H_{29}O_4$ (M + H)$^+$ 333.2066, found 333.2061.

19-Hydroxysarcophine (**10**): colorless powder from EtOH, mp 86−88 °C, $[\alpha]_D^{25}$ +6.3 (*c* 0.12, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH)

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224 (2.70), 248 (2.69), 270 (2.54) nm; IR ν_{max} ($CHCl_3$) 3467, (OH), 3020−2850, 1745 (C═O) 1659, 1602, 1235, 880 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRFABMS *m*/*z* calcd for $C_{20}H_{29}O_4$ $(M + H)^+$ 333.2066, found 333.2049.

9**β**-Hydroxysarcophine (**11**): colorless needles from EtOH, mp 98−99 °C, $[\alpha]_D^{25}$ +181.5 (*c* 0.2, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 225 (2.52), 246 (2.48), 267 (2.43) nm; IR ν_{max} ($CHCl_3$) 3480, (OH), 3012−2858, 1747 (C═O) 1678, 1587, 1236, 880 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRFABMS *m*/*z* calcd for $C_{20}H_{29}O_4$ $(M + H)^+$ 333.2066, found 333.2076.

5**β**-Hydroxysarcophine (**12**): colorless needles from EtOH, mp 100−102 °C, $[\alpha]_D^{25}$ +39.7 (*c* 0.11, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 226 (2.70), 249 (2.74), 269 (2.51) nm; IR ν_{max} ($CHCl_3$) 3495, (OH), 3090−2850, 1743 (C═O) 1644, 1463, 1230, 875 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; HRFABMS *m*/*z* calcd for $C_{20}H_{29}O_4$ $(M + H)^+$ 333.2066, found 333.2053.

10**β**-Hydroxysarcophine (**13**): colorless needles from EtOH, mp 96 °C, $[\alpha]_D^{25}$ −17.1 (*c* 0.12, $CHCl_3$); UV λ_{max} (log ϵ) (MeOH) 226 (2.71), 250 (2.69), 270 (2.49) nm; IR ν_{max} ($CHCl_3$) 3467, (OH), 3019−2840, 1747 (C═O) 1659, 1602, 1235, 880 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; HRFABMS *m*/*z* calcd for $C_{20}H_{29}O_4$ $(M + H)^+$ 333.2066, found 333.2062.

10**α**-Hydroxysarcophine (**14**): colorless needles from EtOH, mp 94−96 °C, $[\alpha]_D^{25}$ +26.4 (*c* 0.13, $CHCl_3$); UV λ_{max} nm (log ϵ) (MeOH) 226 (2.70), 249 (2.66), 266 (2.51); IR ν_{max} ($CHCl_3$) 3467, (OH), 3020−2831, 1747 (C═O) 1678, 1603, 1235, 875 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; HRFABMS *m*/*z* calcd for $C_{20}H_{29}O_4$ $(M + H)^+$ 333.2066, found 333.2087.

7,8-Sarcophine diastereoisomer (**15**):[27] The EtOAc extract of *S. glaucum* was subjected to flash silica gel 60 column chromatography, using a *n*-hexane/EtOAc system. The fraction displaying two sets (1:1) of sarcophine ^1H NMR signals (177 mg) was subjected to prep TLC with $AgNO_3$-impregenated Si gel G_{254}, using cyclohexane−EtOAc (4:1) to afford **3** (82.5 mg, R_f 0.85) and **15** (76.5 mg, R_f 0.74). Compound **15** was shown to be natural since it was detected by TLC in the fresh EtOAc extract of the animal.

Anchorage-Independent JB6 Cell Transformation. The assay for promotion to anchorage independence was performed as described previously by Colburn et al.[25] Briefly, 60 mL of 2× medium were prepared by mixing 40 mL of 2× DMEM, 10 mL of phosphate-buffered saline (PBS), 10 mL of FBS, and gentamicin (50 μg/mL mixture was prepared by adding 60 mL of the 2x media to 40 mL of 1.25% agar at 44 °C, poured into 6-well plates (3 mL/well), and allowed to set (0.5% agar). JB6 cells (P^+, 41-5a; tumor promotion sensitive) grown to about 70% confluency were washed with Ca^{2+}, Mg^{2+}-free PBS and harvested from tissue culture plates by treatment with 0.05% trypsin−EDTA. For each test condition, a 2.5 mL aliquot of the cell suspension (3×10^4 cells/mL) was added to 5 mL of the agar-media mixture, followed by the addition of 7.5 μL of each test agent, TPA (final concentration, 10 nM) or TPA plus test agent. This mixture was layered (1 mL of 0.33% agar/well) onto the agar plates and incubated at 37 °C in a humidified 5% CO_2/air chamber. Colony formation was determined after 14 days of incubation. For each treatment group, the average number of colonies (clusters containing 16 or more cells) were counted in three randomly chosen cm^2 areas on each of three plates.

Evaluation of Growth Inhibitory Potential with JB6 Cells. Test compounds were assessed for potential to inhibit JB6 cell proliferation as described previously.[31] In brief, JB6 cells were distributed in 96-well plates and treated with various concentrations of test compounds originally dissolved in 10% EtOH. Following an incubation period of 3 days, cells were fixed by treatment with 50% aqueous trichloroacetic acid and stained with 0.4% sulforodamine B dissolved in 1% aqueous acetic acid. After washing with 1% acetic acid, cellular bound dye was solubilized by addition of 10 mM Tris buffer, and absorption was monitored at 515 nm. Results were compared with those of zero-day controls and controls incubated 3 days with solvent alone. 6-Thioguanine was used as a positive control.

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Supporting Information Available: ^1H and ^{13}C NMR, HMQC, and HMBC spectra of **4**−**14** (except HMBC of **9**), tables of crystal and structure refinement data, bond lengths and angles, anisotropic displacement parameters, and hydrogen coordinates and isotropic displacement parameters for **3** and **5** and atomic coordinates and equivalent isotropic displacement parameters for **3**, and an ORTEP diagram for 3 (53 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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